NOTE TO FILE
BNF0060
October 20, 1999

Subject: Cantaloupe with a Modified Fruit Ripening Phenotype (Lines A and B)

Keywords: Cantaloupe, *Cucumis melo*, lines A and B, S-adenosylmethionine (SAM), S-adenosylmethionine hydrolase (SAMase), *sam-k* gene, *E. coli* bacteriophage T3, kanamycin resistance, *kan*^{r}, neomycin phosphotransferase II (nptII)

Background

In a submission dated May 5, 1999, Agritope, Inc., provided FDA with its summary of the nutritional and safety assessment of a bioengineered cantaloupe containing the gene encoding the enzyme S-adenosylmethionine hydrolase (SAMase). The firm started consulting with the agency regarding this product on October 20, 1998.

Intended Effect of the Genetic Modification

According to Agritope, the intended technical effect of the genetic modification of the cantaloupe, *Cucumis melo*, is the modification of the ripening phenotype. This modified fruit ripening phenotype results in greater production flexibility and prevents losses due to overripe fruit in the field, packaging, handling and distribution systems. Agritope accomplished this by inserting the gene encoding SAMase (*sam-k*) derived from the *E. coli* bacteriophage T3 (under the control of a promoter that directs fruit-specific, ethylene sensitive expression) into the cantaloupe genome. SAMase degrades S-adenosylmethionine (SAM), which is the penultimate precursor in the ethylene biosynthetic pathway. Ethylene is the plant hormone that promotes fruit ripening.

Introduced Genetic Material

Agritope used the *Agrobacterium tumefaciens* T-DNA transformation system to introduce the *sam-k* gene (with the synthetic combination of the *E8* and *E4* gene promoters from the tomato and the terminator from the untranslated 3' region of the nopaline synthase gene (nos) from *A. tumefaciens*) and the *kan*^{r} gene from the transposon Tn*5* (with a promoter modified for constitutive expression from the *RE4* gene from raspberry and the terminator from the untranslated 3' region of gene *7* from *A. tumefaciens*). This *sam-k* gene was modified to contain a consensus eukaryotic translation initiation site by altering the nucleotide sequence surrounding the ATG start codon. The inserted DNA also contains sequences from the right and left border regions of T-DNA from *A. tumefaciens*. Agritope used Southern analysis of the initial transformant and progeny of each line to demonstrate the integration of the T-DNA at a single locus and the stability of the genetic insert over several generations. Agritope used PCR analysis

of each line to demonstrate that the *kan*^{r} gene, as well as the *E4/E8* and *RE4* promoters were intact, and that sequences outside of the T-DNA borders, present on the transforming plasmid, were not integrated into the *Cucumis melo* genome.

Safety of Expressed Proteins

Two new proteins, namely SAMase, and the *kan*^{r} gene product, neomycin phosphotransferase II (nptII), are expressed in the transgenic cantaloupe lines A and B. The nptII enzyme is approved for use as a selectable marker in the production of transgenic cotton, tomato, and rapeseed (21 CFR 173.170 and 573.130). The nptII protein is not known to be toxic and is known to be rapidly degraded under simulated gastric conditions. Agritope describes a feeding study in mice in which a single dose of 5 grams (g) nptII per kilogram (kg) of body weight showed no effects. Agritope cites a U.S. Environmental Protection Agency document which derived the Maximum Hazard Dose for nptII as 5g/kg. Using western analysis to detect and quantify the nptII protein, Agritope determined the average concentration in ripe fruit for line A is approximately 77 picograms (pg) per microgram (ug) of protein (0.0076% of total protein), and in line B 16 pg/ug protein (0.0016% of total protein). Based on these data Agritope calculates that an individual would need to consume more than 450,000 kg of cantaloupe from line A and more than 2 million kg of line B to achieve a 5 g/kg dose. Agritope therefore concluded that the nptII protein is safe for use in cantaloupe.

According to Agritope, stable integration of the *sam-k* transgene in the cantaloupe results in the production of SAMase in a fruit specific and temporally regulated manner. Agritope measured the production of ethylene in full slip or mature fruit in the transgenic lines compared to their non-transgenic controls, and found that ethylene production is decreased in the transgenic lines. Agritope used western analysis to demonstrate that SAMase is not expressed in immature fruit or plant tissues other than mature fruit.

Agritope used western blot analysis to calculate the level of SAMase in fruit in the transgenic cantaloupe lines at about 32 pg/ug of protein for line A and 22 pg/ug for line B. This results in a SAMase protein concentration of approximately 0.003% and 0.002% of the total protein found in ripening cantaloupe of lines A and B, respectively. Agritope states that the SAMase protein is ubiquitous in the digestive tract of human beings due to its association with coliphage T3 infections of resident *E. coli* suggesting that background exposures to this protein are ongoing.

Agritope stated that neither the mode of action of SAMase, nor the by-products of the reaction catalyzed by SAMase, raises any safety concern. Database searches did not demonstrate any homology to any protein (and thus to any toxin or allergen) in the three major protein databases (University of Geneva protein sequence databank (Swiss-Pro-31), the Protein Identification Resource sequence databank (PIR46) and the GENESEQ Patented amino acid sequence databank (A-Gene Seq. 21)).

Agritope concluded that the consumption of SAMase in cantaloupe fruit will not raise any allergenicity concerns because the enzyme does not have characteristics commonly attributed to food allergens. Agritope stated that SAMase does not contain the necessary sequence information needed for transport of the protein to the subcellular locations where glycosylation takes place; SAMase protein is rapidly inactivated by incubation under simulated gastric conditions; SAMase enzyme activity is not heat stable; the low concentration of SAMase in ripe cantaloupe fruit further supports a conclusion of no concern with respect to allergenicity.

Agritope has previously completed a consultation process on a new tomato variety that was engineered to have a modified ripening phenotype through fruit-specific expression of a transgene encoding the enzyme SAMase (BNF 0014). The consultation was successfully completed in March 1996 as the agency found no outstanding safety or regulatory issues regarding the use of SAMase protein in tomato.

Compositional Analysis: Nutrients and Endogenous Toxicants

Agritope concluded that cantaloupe lines A and B are different from the non-transformed parental lines only in the presence of nptII and SAMase and their intended technical effects. Agritope stated that the fruit derived from lines A and B did not differ from fruit derived from the non-transgenic controls in horticultural characteristics and fruit quality: external firmness, internal firmness, soft spots, stem end quality, presence of mold, and sugar accumulation. Agritope also analyzed for total carotenes, vitamin C, total carbohydrates, total sugar, percent protein, sodium, calcium, iron, and found that transgenic lines did not differ from their non-transformed controls in any of these constituents. Agritope stated that all values obtained for these parameters, except vitamin C and total sugars, fall well within the range normally reported for fresh cantaloupe. The values obtained for vitamin C and total sugars fall below the range normally reported, but were comparable in the parental (non-transgenic controls) and transgenic cantaloupe lines.

Finally, Agritope stated that Cucurbitacin B and E alkaloids are the primary cucurbitacins found in *Cucumis melo*. Cucurbitacin B and E are readily detectable by taste at levels as low as 1 to 10 parts per billion. Agritope finds that both the transgenic lines A and B as well as the non-transgenic lines from which they were derived are non-bitter.

Conclusions

Agritope has concluded that cantaloupes from transformed lines A and B are safe as food and do not differ significantly from traditionally developed cantaloupes except that of the intended technical effects of the *sam-k* and *kan*^{r} genes. At this time, based on Agritope's description of its data and analyses, the Agency considers Agritope's consultation on transgenic cantaloupe lines A and B to be complete.

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